Applications of Molecular Imaging to Drug Discovery and Development.

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Background:

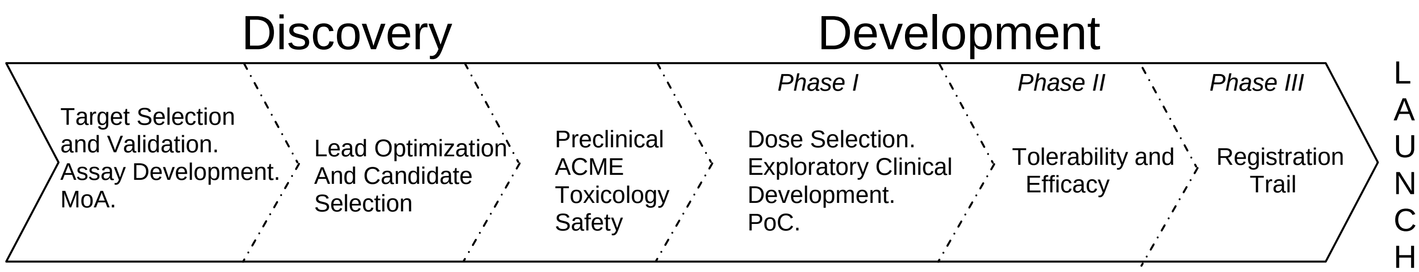
In the last few years techniques of molecular imaging have become established in the pharmaceutical industry. Small animal imaging systems that were once only found in dedicated academic departments are now core facilities within the industry supporting both drug discovery and early development. The imaging technologies available to the experimentalist for small animal research include magnetic resonance imaging (MRI), computer tomography (microCT), positron emission tomography (microPET), ultrasound (US) and optically imaging (OI) techniques. Since no one technique has the capability to address all the questions posed by the investigator, most imaging facilities in moderate to large pharmaceutical companies have developed a common technical center with the range of imaging modalities and necessary peripheral support, including dedicated resources for image analysis and data management.

The increased demand for molecular imaging in drug discovery and development has been influenced by several converging factors. Firstly, the availability of techniques for the genetic manipulation of laboratory mice together with the unraveling of the human, and now mouse, genomes provides the experimentalist the unprecedented ability to generate models that recapitulate certain aspects of the human disease. Non-invasive molecular imaging techniques that can study disease progression and monitor the effects of therapeutic interventions in these animal models are essential. Secondly, in addition to the expanding preclinical demand for non-invasive imaging techniques for basic research the utility of medical imaging in clinical drug development, enabling first in human studies, is being realized and supportive preclinical validation is required. Applications of molecular imaging can be found at every stage of the drug discovery and development process and their impact includes:

(a) Expedite the identification of lead compounds
(b) Identify novel targets
(c) Understand pharmacokinetics and distribution
(d) Elimination of compounds due to inactivity or toxicity
(d) Identify the pharmacological mode-of-drug-action
(e) Validate clinical biomarkers

The Stages of Drug Development:

The specific approach to drug discovery and subsequent development depends on number factors including the therapeutic area, molecular entity (biologics or synthetic), target patient population etc., but in general the process can be viewed as several sequential steps from basic research to product launch:



This lecture will provide an overview of the applications of molecular imaging with specific example of the impact at each stage of the process.

Discovery:

In essence, drug discovery consists of understanding the molecular nature of the disease and identifying targets, often genes or proteins, responsible for the formation and progression of the disease. Developing animal models that resemble the clinical conditions is an important part of the early research, enhancing our understanding of the pathophysiology and providing an in vivo system to evaluate the efficacy of putative drug candidates.

As the genotype of human cancer is identified, animal models with these identical mutations can be recapitulated. For example, the K-ras oncogene is frequently mutated in human lung adenocarcinoma and mice containing the conditional mutation (KrasG12D) produce lung tumors that resemble the human pathology, both genetically and histologically (1). Techniques of gene profiling have been applied to these model systems to identify potential therapeutic targets. For example, in the K-ras$^{(G12D)}$ mutant mice several cathepsins proteases are strongly unregulated in this system (2). Proteases have been implicated in tumor metastasis and invasion and as such represent a target for therapeutic intervention. Although these eloquent model systems can be used to identify proteases that are over expression at the RNA level, confirmation of causality can only be obtained if target inhibition reduces tumorigenicity. To this end, high affinity inhibitors could be tested in these models using protease activity as the readout. Fluorescently labeled near infrared probes that are sensitive to protease activity and can be detected in vivo have been developed. These molecular imaging probes are activated upon enzymatic cleavage by proteases. The fluorochromes are held in close proximity with one another on a peptide backbone comprising the target sequence. In this configuration the fluorochrome are effectively quenched and their fluorescence reduced. Upon enzymatic cleavage the resulting separation of the fluorochromes result in increase in fluorescence (3). These “smart probes” are often designed to fluorescence in the near infrared (NIRF) to enable in vivo imaging – maximizing tissue penetration whilst reducing auto-fluorescence. These probes have been developed to a number of proteases with known peptide cleavage sequences including cathepsin B and D, thrombin and caspase-3 and have been used to demonstrate specific proteases activity in a number of models of disease including cancer and rheumatoid arthritis. The major advantages of using such an in vivo molecular imaging approach for drug screening is that the fluorescence readout of drug action measures not only target inhibition but incorporates metabolism and pharmoacokinetics, thus accelerating the identification and validation of lead candidates.

One of the major challenges to developing successful therapeutic is identifying casual pathways and “drugable” targets in the deluge of information from microbiological research. Recently techniques of RNA silencing have emerged as one of the most powerful tools for functional gene analysis (4, 5). The introduction of the siRNA into cells can interfere with function of the endogenous gene and protein expression due to a sequence specific interaction between the siRNA and mRNA. Monitoring the effect of gene ablation can be performed in vivo with bioluminescence imaging by using firefly-luciferase fusion construct to the target gene of interest. For example, the combination of in vivo RNA interference and molecular imaging has been used to study the in vivo consequences of RNA interferences-mediated ablation of MDR1 P-glycoprotein (6). In cancer over expression of MDR1 confers resistance to chemotherapy by acting as an energy-dependant efflux transporter. Selective blockage of MDR1 in vivo, as monitored by whole body bioluminescence, can be used to study drug efficacy and resistance. Changes in bioluminescence signal intensity reflects the protein level and can be used to monitor the influence of drug resistance on novel cancer therapies prior to clinical trials.

The conventional paradigm for developing targeted inhibitors in oncology is to use a high-affinity proof-of-concept (PoC) inhibitor with acceptable metabolic properties for key target validation experiments. However, this approach is resource and time intensive requiring substantial medicinal chemistry, toxicity and PK support. An alternative approach that has recently been implemented is to exploit the RNA interference techniques. For example, mutations in BRAF occur with high frequency in melanomas (70%), papillary thyroid carcinomas, serous ovarian cancers, and colorectal - implicating BRAF in tumorigenicity. Validation of BRAF as a target was performed using inducible short-hairpin RNA in xenograft models and using luciferase as a reporter construct. In vivo bioluminescence imaging of a metastatic melanoma model showed that conditional BRAF suppression slowed systemic tumor growth (7). Combining techniques of molecular imaging with RNAi provides a toolbox for rapid validation of putative therapeutic targets.

Development:

Drug development starts once the pharmacological target has been validated and leads candidates selected. Before moving into human studies as part of the early clinical development extensive preclinical testing is required to support investigational new drugs filing (IND) with the FDA. This early development phase is a critical decision point before moving into full development, and in addition to detailed toxicology studies includes PK (pharmacokinetics) and PD (pharmacodynamics) defines the drugs absorption, distribution, metabolism and excretion (ADME) characteristics. It has been estimated that there is a failure rate of up to 40 % of lead development candidates due to unpredicted metabolism or PK. Here again techniques of molecular imaging, in particular PET, can impact the drug development process. For biologics, like anti-body based therapies, species cross reactivity often restricts ADME studies to higher species. To assist with pre-IND PK studies conjugation of antibodies with radiolabeled ligands are used to monitor tissue distribution and accumulation. These studies have the advantage that they can be performed across species including rodents, NHP and, after the appropriate safety testing, humans. An extensive literature now exists for labeled antibodies and engineered fragments in rodents and humans. Indeed such an approach has been incorporated into the early clinical development, including optimization of dosing regimens and patient selection, for targeted therapies that use radio immune conjugates (8).

Human PET studies involve microdosing (i.e. a dose that is several fold lower than that required to produce a measurable pharmacologic effect) have limited human exposure. As a result, the FDA has introduce alternative regulatory requirements that, while maintaining needed human subject protection, involve fewer resources than customary for therapeutic IND (9). This can accelerate drug development by identifying (or excluding) lead candidates based on their PK/PD properties and can results in dramatic cost saving and avoids exposure of humans to exploratory drugs that might not be effective. The use of microdosing PET studies has successful been incorporated into PoC studies for a number of potential therapies targeting the CNS disease. Optimization of dose and delivery can be challenging in the CNS where the plasma concentration is a poor predicators of tissue accumulation and receptor occupancy. PET imaging of isotope labeled ligand to a specific receptors that can be displaced by the drug under investigation have been used to confirm on target effects as well as deriving the relationship between plasma levels and target tissue concentration. For example, in the development of a novel anti-emetic a radiolabeled tracer specific to the NK1 receptors in the brain was used to establish the plasma dose relationship to receptor occupancy in healthy volunteers (10). The data demonstrated that high levels of receptor occupancy were required to achieve optimal effect and the dosing level could be adjusted accordingly for the subsequent clinical trials.

Early Clinical Development:

Once the drug target has been validated, a dose selected and extensive safety testing in both laboratory based assay and animals has been obtained, PoC studies are perform in humans. PoC studies are performed in a small group of patients or healthy volunteers to verify the mechanism of action and to gain insight into whether the new drug entity is likely to be efficacious in human disease. These PoC studies are not intended to substitute for large registration trails (phase III trials necessary for approval by the FDA), rather are intended to influence decisions prior to embarking on large extensive phase III trails using conventional and accepted clinical endpoints. A recent example of a successfully PoC studies that generate early and compelling clinical data was using the kinase inhibitor imatinimb mesylate (Gleevec™) to treat soft tissue sarcoma (11). Dramatic results were observed on FDG-PET scans performed within several days after onset of treatment. The FDG-PET response preceded any detectable change on CT exam or clinical score thus providing early evidence of on-target drug response. Though the use of imaging biomarkers for the assessment of study endpoints, PoC trials can usually be completed in less than a year. Further technological advances in field of molecular iamging will increase both the sensitivity and specificity of imaging biomarker for the disease state and will gain increasing acceptance for early drug development (12).

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